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Microbial loads and incidence of food-borne indicator bacteria in most popular indigenous fermented food condiments from middle-belt and southwestern Nigeria

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The food indicator bacteriological quality of 1 501 samples of most-consumed Nigerian fermented food condiments (*iru* n = 1 125, *ogiri* n = 148, *okpehe* n = 113 and *ugba* n = 115), randomly obtained from various markets in eleven major cities of Nigeria, was determined. A total of 472 strains of *Staphylococcus aureus* and 3 556 Gram-negative indicator bacterial strains, *Escherichia coli* (863 [24.3%]), *Klebsiella pneumoniae* (671 [18.8%]), *Proteus mirabilis* (591 [16.6%]) and *Pseudomonas aeruginosa* (374 [10.5%]) were isolated. The other isolated bacterial species were *Klebsiella aerogenes* (299 [8.4%]); *Citrobacter aerogenes* (264 [7.4%]); *Enterobacter aerogenes* (227 [6.4%]); *Shigella dysenteriae* (168 [4.7%]), *Shigella flexneri* (60 [1.7%]) and *Shigella sonnei* (39 [1.1%]). The most frequently recovered bacterial species from *iru* were *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa*, while *E. coli*, *K. pneumoniae* and *P. mirabilis* were the most recovered from *ogiri*. Similarly, *E. coli* and *K. pneumoniae* were the most recovered species from *okpehe* and *ugba* samples, indicating lack of process efficiency of the cottage-produced fermented food products.

Key words: Coliforms, condiments, fermentation, food-borne pathogens, food poisoning, food safety, HACCP, indicator organisms, indigenous, microbial quality.

INTRODUCTION

Fermented foods are defined as palatable products, which are prepared from raw or heated materials and which acquire their characteristic properties by a process that involves microorganisms (Buckenhuskes, 1993). They are essential parts of diets in all parts of the world, and since Africa is a vast continent grappling with the problem of feeding its teeming population, fermented foods and beverages constitute a major portion of peoples' diet.

Fermented condiments give pleasant aroma to soups and sauces in many countries, especially in Africa and India where protein calorie malnutrition is a major problem (Sarkar et al., 1993). They also have great potential as key protein and fatty acid sources, and are good sources of gross energy. Therefore, condiments are basic ingredients for food supplementation and their socio-economic importance cannot be over emphasized. In Africa, many proteinaceous oily seeds are fermented to produce food condiments (Odunfa, 1985; Sanni and Ogbonna, 1991; Baird- Parker, 1994; Leejerajumnean et al., 2000; Azokpota et al., 2006; Ogunshe et al., 2006, Folarin et al., 2007; Ogunshe et al., 2007; Ogunshe et al., 2008; Ogunshe and Ogundimu, 2008). *Iru* (also known as *dadawa*/*dawadawa*) is a fermented product of the African locust bean (*Parkia biglobosa*). It serves as an important food condiment in Nigeria, and many other countries of west and central Africa (Campbell–Platt, 1984) . Another vegetable protein that is common in West Africa is *ogiri*, a fermented condiment from melon (*Citrullus vulgaris*) seeds. It is a popular strong-smelling food condiment also consumed by the Ijebu and Ondo tribes in the forest zone of southwestern Nigeria. *Ugba* is a Nigerian fermented vegetable protein from the African oil bean (*Pentaclethra*

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macrophyla) (Obeta, 1982; Ejiofor et al., 1987), popularly consumed in the eastern parts of the country, while *okpehe*, also known as *afiyo*, is a strong-smelling fermented condiment from *Prosopis africana* and highly popular in the middle-belt of Nigeria (Ogunshe et al., 2006).

Among the requirements for foods to be of good sanitary quality, they must be shown to be free of hazardous microorganisms, or those present should be below minimum safe limits. Contamination of foods by disease-causing microorganisms has, however, been known and studied since around 1880.

Since that time, numerous instances of food-borne diseases have been recorded, and in spite of our knowledge of microbiology, as well as the implementation of safety procedures such as hazard analysis critical control point (HACCP), the worldwide incidence of food poisoning is increasing (Baird-Parker, 1994). Thus, food safety remains a major challenge to food producers and to legislators endeavouring to provide adequate consumer protection. This study attempts to highlight some of the food safety challenges associated with traditional fermented condiments in the middle-belt and south-western Nigeria by determining the occurrence of pathogenic food indicator bacteria in the most- consumed Nigerian fermented condiments, so that further investigations can consider the improvement of such indigenous foods.

MATERIALS AND METHODS

Sample collection

Nigeria is presently divided into 36 states and Abuja, the Federal capital of Nigeria. Molds of each of the locally fermented condiments (*iru*, o*giri* , *ugba* and *okpehe*), weighing between 100-250 g, used in this study, were randomly obtained from local markets in 11 major cities (Lagos, Ibadan, Ijebu-Ode, Abeokuta, Ilorin, Benin, Ore, Gboko, Lokoja, Abuja, Okenne) from eight sampling states (Lagos, Edo, Ogun, Oyo, Kwara, Ondo, Kogi and Benue, as well as the Federal capital territory) over a period of 28 months. These sample locations comprised of the middle-belt and southwestern Nigeria where the sampled condiments are indigenous, very popular and in high demand. The condiments were purchased as usually sold, that is, wrapped in certain leaves and tied with leaf strings. The samples from Lagos, Ibadan, Ijebu-Ode and Abeokuta were collected monthly, while those from Ilorin, Benin, Ore, Gboko, Lokoja, Abuja and Okenne were collected bi-monthly. The purchased condiments were separately packaged in polythene bags and transported to the laboratory for microbial analyses.

pH determination

Ten grams of each condiment was dissolved in 100 ml of sterile distilled water and the pH of the homogenate of each condiment sample was determined using a Pye unicam pH meter (Unicam 9450 model) equipped with a glass electrode. Determinations were done in triplicates.

Culture media

Staphylococcus aureus strains were isolated on mannitol salt agar (MSA, Lab M), while the Gram-negative indicator bacteria were isolated on MacConkey agar (MCC, Lab M), eosin methylene blue agar (EMB, Lab M) and violet red bile glucose agar (VRBG, Lab M). Tentative identification of pure cultures in triple sugar iron agar (TSI, Lab M) slants was performed, and pure cultures were maintained on brain heart infusion agar (BHI, Oxoid) slants at 4^{o}C.

Bacterial characterization

Isolation of *S. aureus* and Gram-negative indicator bacteria from the fermented condiments was performed by selective pour-plating culture procedures and by standard coliform test methods. Representatives of each different colony type observed were sub-cultured on BHI agar and MacConkey agar plates until pure cultures were obtained. The colony, cell morphology and standard biochemical tests of each pure culture were determined according to bacterial taxonomical methods (Holding and Collee, 1972; Seeley and Van Denmark, 1972; Harrigan and McCance, 1976; Cheesbrough, 1998, 2000).

Haemolysis test

All the indicator bacterial isolates were tested for their haemolytic characteristics on blood agar containing 10% human blood in blood agar base (Lab M, UK). Variable haemolytic properties that permit differentiation of the species of bacteria were determined (Prescott et al., 2005).

RESULTS

One thousand one hundred and twenty five *iru* / *dadawa* / *dawadawa* samples from *P. biglobosa;* 148 *ogiri* samples from *C. vulgaris;* 113 *okpehe* samples from *P. africana* and 115 *ugba* samples from *P. macrophylla* were microbiologically analyzed in this study. The pH of the fermented condiments was between 7.0 and 9.0, indicating a neutral to slightly alkaline pH range. More of the *okpehe* (35.4%), *ogiri* (33.8%) and *ugba* (31.3%) samples had a pH of 8.0, while 20.6% of *iru* samples had a pH of 8.0 (Table 1).

Sixty six percent of the *iru* samples were total coliform-positive, while 82.4, 85.0 and 93.9% of the *ogiri*, *ugba* and *okpehe* samples were total coliform-positive, respectively, producing both acid and gas in sterile MacConkey broth at 35^{o}C after 24 h of incubation. A total of 4 028 (n = 472 *S. aureus*; n = 3 556 Gram-negative) indicator bacteria were obtained from the fermented condiments, which were characterized as *E. coli* (863 [24.3%]), *K. pneumoniae* (671 [18.8%]), *P. mirabilis* (591 [16.6%]), *P. aeruginosa* (374 [10.5%]), *E. aerogenes* (227 [6.4%]), *K. aerogenes* (299 [8.4%]) and *C. aerogenes* (264 [7.4%]). Other identified bacteria were *S. dysenteriae* (168 [4.7%]), *S. flexneri* (60 [1.7%]) and *S. sonnei* (39 [1.1%]).

A relatively high prevalence rate of indicator bacteria,

Table 1. pH and total coliform profiles of the analysed fermented food condiments.

Fermented condiments					
pH	***Iru***	***Ogiri***	***Okpehe***	***Ugba***	**No./ (%)of samples**
7.0	244(21.7)	25(16.9)	40(35.4)	36(31.3)	345(23.0)
7.5	221(19.)	29(19.6)	07(6.2)	10(8.7)	267(17.8)
8.0	232 (20.6)	50(33.8)	40(35.4)	36(31.3)	358(23.9)
8.5	205(18.)	22(14.9)	20(17.7)	10(8.7)	257(17.1)
9.0	223(19.)	22(14.9)	06(5.3)	23(20.0)	274(18.3)
Total no of samples	1125	148	113	115	1501
Total coliform +ve samples	743(66.0)	122(82.4)	96(85.0)	108(93.9)	1069(71.2)

Values in parenthesis are in %

Table 2. Prevalence and sampling sources of indicator bacterial isolates from fermented *iru* samples.

Indicator bacteria	Total number of isolates from samples	% of isolates from samples	Contaminated sample sources	cfu/g of indicator bacteria^
Citrobacter aerogenes	630	56.0	[a1, a2, a3, a4, a6, b1]	2.04×10^{4} - 9.1×10^{6}
Escherichia coli	935	83.1	[a1, a2, a3, a4, a5, a6, a7, a8, b1, b2, b4]	2.71×10^{7} - 1.23×10^{8}
Enterobacter aerogenes	251	22.3	[a2, a4, a5, a7, b1, b2, b3, b4]	2.11×10^{6}-1.95×10^{8}
Klebsiella aerogenes	383	34.0	[a1, a2, a3, a5, a6, b2, b4]	2.03×10^{6} -1.15×10^{7}
Klebsiella pneumoniae	1031	91.6	[a1, a2, a3, a4, a5, a6, a7, b1, b2, b3, b4]	2.18×10^{7} - 9.4×10^{8}
Proteus mirabilis	692	61.5	[a1, a2, a3, a4, a5, a6, a7, b1, b2, b3, b4]	* - 2.00×10^{5}
Pseudomonas aeruginosa	674	59.9	[a1, a2, a3, a4, a5, a6, a7, b1, b2, b3, b4]	1.12×10^{5} - 7.5×10^{7}
Shigella dysenteriae	517	46.0	[a1, a2, a3, a4, a5, a6, a7, b1, b2, b3, b4]	1.23×10^{5} -1.03×10^{6}
Shigella flexneri	409	36.4	[a2, a3, a5, a6, a7, b1, b2, b3, b4]	1.04×10^{4}- 5.6×10^{5}
Shigella sonnei	153	13.6	[a1, a3, a4, a5, a6, a7, b1, b2, b3, b4]	9.6×10^{5} - 8.1×10^{4}
Staphylococcus aureus	855	76.0	[a1, a2, a3, a4, a5, a6, a7, b1, b2, b3, b4]	2.37×10^{5} - 1.01×10^{4}
Total no of samples determined	1125			

* Swarming plates.
^ Calculated for positive samples only. Values in parenthesis are the sampling sources (a1 = Lagos, a2 = Ibadan, a3 = Ijebu-Ode, a4 = Abeokuta, a5 = Ilorin, a6 = Benin, a7 = Ore, b1 = Gboko, b2 = Lokoja, b3 = Abuja, b4 = Okenne)

13.6 - 91.6% in *iru*, 62.8 - 95.3% in *ogiri*, 34.5 - 91.2% in *okpehe*, and 41.7 - 92.2% in *ugba* samples, were recorded, irrespective of the sampling source (Tables 2 - 5). It is indicated from the results obtained in this study that the most recovered bacterial species from *iru* were *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*. *E. coli*, *K. pneumoniae* and *Proteus mirabilis* were the most recovered from *ogiri*, while *E. coli* and *K. pneumoniae* were among the most recovered species from *okpehe* and *ugba* samples (Table 6). All the Gram-negative indicator bacteria and *Staphylococcus* strains obtained in this study were -haemolytic.

DISCUSSION

Iru, *ogiri*, *okpehe*, and *ugba* are the most popular Nigerian indigenous fermented condiments (Ogunshe et al., 2007) and the pH of these condiments being slightly alkaline agrees with some earlier reports, which all recorded a slightly alkaline to alkaline pH in fermented food condiments from vegetable proteins (Hesseltine**,** 1965; Odunfa, 1985; Baird- Parker, 1994; Ogunshe et al., 2006, 2007). The increase in pH is generally due to the production of ammonia and amines, and is quite common with the fermentation of vegetable proteins during the

Table 3. Prevalence and sampling sources of indicator bacterial isolates from fermented *ogiri* samples

Indicator bacteria	Total number of isolates from samples	% of isolates from samples	Contaminated sample sources	cfu/g of indicator bacteria^
Citrobacter aerogenes	131	88.5	[a1, a2, a3, a4, a7]	1.29×10^{6} - 2.37×10^{9}
Escherichia coli	138	93.3	[a1, a2, a3, a4, a7]	1.73×10^{8} - 2.06×10^{9}
Enterobacter aerogenes	115	77.7	[a1, a2, a3, a4, a7]	2.01×10^{6} - $2\ 48 \times 10^{9}$
Klebsiella aerogenes	99	66.9	[a1, a2, a3, a4, a7]	1.33×10^{5} -2.10×10^{8}
Klebsiella pneumoniae	141	95.3	[a1, a2, a3, a4, a7]	2.13×10^{8} - 9.4×10^{9}
Proteus mirabilis	116	78.4	[a1, a2, a3, a4, a7]	* - 2.0×10^{5}
Pseudomonas aeruginosa	114	91.2	[a1, a2, a3, a4, a7]	1.03×10^{6} - 1.28×10^{8}
Shigella dysenteriae	95	64.2	[a1, a2, a3, a4, a7]	1.01×10^{7} - 1.03×10^{8}
Shigella flexneri	109	73.6	[a1, a2, a3, a4, a7]	9.40×10^{6}- 3.5×10^{7}
Shigella sonnei	102	68.9	[a1, a2, a3, a4, a7]	4.7×10^{6} x 7.4×10^{7}
Staphylococcus aureus	93	62.8	[a1, a2, a3, a4, a5, a6, a7, b1, b2, b3, b4]	1.12×10^{4} -1.01×10^{5}
Total no of samples determined	148			

* Swarming plates.
^ Calculated for positive samples only. Values in parenthesis are the sampling sources (a1 = Lagos, a2 = Ibadan, a3 = Ijebu-Ode, a4 = Abeokuta, a5 = Ilorin, a6 = Benin, a7 = Ore, b1 = Gboko, b2 = Lokoja, b3 = Abuja, b4 = Okenne).

Table 4. Prevalence and sampling sources of indicator bacterial isolates from fermented *okpehe* samples

Indicator bacteria	Total number of isolates from samples	% of isolates from samples	Contaminated sample sources	cfu/g of indicator bacteria^
Citrobacter aerogenes	103	91.2	[a1, a2, a5, b1, b2, b3, b4]	1.25×10^{4} - 1.31×10^{5}
Escherichia coli	102	90.3	[a1, a2, a5, b1, b2, b3, b4]	1.21×10^{5} - 1.15×10^{5}
Enterobacter aerogenes	98	86.7	[a1, a2, a5, b1, b2, b3, b4]	1.17×10^{4}-1.04×10^{5}
Klebsiella aerogenes	75	66.4	[a1, a2, b1, b2, b3, b4]	1.18×10^{4} -2.01×10^{5}
Klebsiella pneumoniae	103	91.2	[a1, a2, a5, b1, b2, b3, b4]	1.23×10^{5} - 1.69×10^{5}
Proteus mirabilis	103	91.2	[a1, a2, a5, b1, b2, b3, b4]	* - 1.0×10^{5}
Pseudomonas aeruginosa	93	82.3	[a1, a2, a5, b1, b2, b3, b4]	1.31×10^{5}- 1.25×10^{5}
Shigella dysenteriae	87	77.0	[a1, a2, a5, b1, b2, b3, b4]	1.42×10^{5} - 1.15×10^{4}
Shigella flexneri	52	46.0%	[a1, a2, a5, b1, b2, b3, b4]	9.1×10^{4} - 1.03×10^{5}
Shigella sonnei	39	34.5	[a1, a2, b1, b2, b3, b4]	8.7×10^{5} x 1.11×10^{4}
Staphylococcus aureus	73	64.6%	[a1, a2, a5, b1, b2, b3, b4]	1.22×10^{5} - 1.65×10^{5}
Total no. of samples determined	113			

*Swarming plates.
^Calculated for positive samples only. Values in parenthesis are the sampling sources (a1 = Lagos, a2 = Ibadan, a5 = Ilorin, a6 = Benin, a7 = Ore, b1 = Gboko, b2 = Lokoja, b3 = Abuja, b4 =Okenne)

hydrolysis of protein (Whitaker, 1978), giving a distinctly ammoniacal smell of the fermented condiments (Leejerajumnean et al., 2000). Alkaline pH created during fermentation of the proteinaceous foods has been documented to make the substrate unsatisfactory for invasion by microorganisms that might cause spoilage of the product (Steinkraus, 1991). This study, therefore, shows that indicator bacterial species were able to withstand the alkaline conditions created by the fermenting process.

The practice that has been in effect for many years and which is continued to be followed is to determine the sanitary quality of foods by their content of certain indicator organisms. It would not be feasible to examine each food or food product for the presence of hazardous organisms. Therefore, the indicators of sanitary quality usually employed for foods include two groups of bacteria, that is, coliforms and enterococci (Jay, 1993). The total coliform groups of bacteria are known as indicator organisms, i.e. organisms whose presence is an index of possible contamination of water and foods by human pathogens (Jay, 1993; Prescott et al., 2005). The traditional methods for detecting coliform bacteria rely upon culturing on a medium that selectively permits the growth of Gram-negative bacteria and differentially de-tects lactose-utilizing bacteria, e.g., using MacConkey or eosin methylene blue media. By using these media and an incubation temperature of 37^{O}C, total coliform bacteria, which include members of the genera *Escherichia*, Ente-

Table 5. Prevalence and sampling sources of the indicator bacterial isolates from fermented *ugba* Samples.

Indicator bacteria	Total number bacteria^ of isolates	% of isolates from samples	Contaminated sample sources	cfu/g of indicator from samples
Citrobacter aerogenes	57	49.6	[a2, a5, a6, b1, b3]	2.23×10^3 - 2.94×10^4
Escherichia coli	101	87.8	[a1, a2, a5, a6, b2, b3]	2.48×10^4 - 1.10×10^6
Enterobacter aerogenes	39	33.9	[a1, a2, a6, b1, b2, b3, b4]	1.14×10^4-2.13×10^6
Klebsiella aerogenes	54	47.0	[a1, a2, a6, a7, b1, b2, b3]	2.05×10^3 -1.32×10^5
Klebsiella pneumoniae	106	92.2	[a1, a2, a5, a6, a7, b1, b2, b3, b4]	2.18×10^5 - 1.61×10^6
Proteus mirabilis	96	83.5	[a1, a2, a5, a6, b1, b2, b3, b4]	* - 1.0×10^4
Pseudomonas aeruginosa	71	61.7	[a1, a2, a5, a6, a7, b1, b2, b3, b4]	1.36×10^3 - 1.13×10^5
Shigella dysenteriae	56	48.7	[a1, a2, a5, a6, b1, b2, b3, b4]	1.02×10^4 - 2.01×10^5
Shigella flexneri	48	41.7	[a1, a2, a6, b1, b2, b3]	1.06×10^4- 1.16×10^4
Shigella sonnei	51	44.3	[a1, a2, b1, b2, b3, b4]	1.04×10^3 x 9.1×10^4
Staphylococcus aureus	86	74.9	[a1, a2, a5, a6, a7, b1, b2, b3, b4]	$1.2.1 \times 10^3$ - 9.4×10^4
Total no. of samples determined	115			

* Swarming plates.
^ Calculated for positive samples only. Values in parenthesis are the sampling sources (a1 = Lagos, a2 = Ibadan, a5 = Ilorin, a6 = Benin, a7 = Ore, b1 = Gboko, b2 = Lokoja, b3 = Abuja, b4 =Okenne).

Table 6. Recovery rates of the indicator bacterial isolates from the fermented food condiments.

Name of isolates	No of indicator bacteria recovered per sample				Total no of indicator bacteria
	Iru	***ogiri***	***okpehe***	***ugba***	
Citrobacter aerogenes	113 (7.68)	96 (8.05)	43 (8.21)	12 (3.27)	267 (7.43)
Escherichia coli	340 (23.1)	320 (26.8)	112 (21.4)	91 (24.8)	863 (24.3)
E. aerogenes	121 (8.22)	24 (2.00)	71 (13.5)	11 (3.00)	227 (6.38)
K.. aerogenes	139 (9.44)	112 (9.39)	43 (8.21)	05 (1.36)	299 (8.40)
K. pneumoniae	236 (16.0)	228 (19.2)	87 (16.6)	120 (32.7)	671 (18.8)
Proteus mirabilis	223 (15.1)	216 (18.1)	91 (17.4)	61 (16.6)	591 (16.6)
P. aeruginosa	185 (12.6)	114 (9.56)	48 (9.16)	27 (7.36)	374 (10.5)
Shigella dysenteriae	91(6.18)	41(3.44)	16 (3.05)	20 (5.45)	168 (4.70)
Shigella flexneri	10 (0.68)	31 (2.60)	08 (1.53)	11 (3.00)	60 (1.68)
Shigella sonnei	14 (0.95)	11 (0.93)	05 (0.95)	09 (2.45	39 (1.10)
Total Gram-negative strains	1472	1193	524	367	3556
S.. aureus	231	146	39	56	472

Values in parenthesis are in %

robacter, *Citrobacter* and *Klebsiella*, among others, can be enumerated (Odunfa, 1985).

The coliform organisms are well established as feacal indicators for water. Their use as indicators of food sanitary quality derives from their successful use for water. The finding of large numbers of these microorganisms in foods and water is taken to indicate faecal pollution or contamination, and since the water-borne diseases are generally intestinal diseases, the existence of pollution is taken to indicate the possibility that the aetiologic agents of these diseases may be present (Chang et al., 1989; Jay, 1993). While the coliforms are generally regarded as being *E. coli* and *E. aerogenes*, it should be noted that the genera *Citrobacter* and *Klebsiella* also come under this functional classification (Prescott et al., 2005).

There have been few documented reports on the prevalence of coliforms and indicator bacterial isolates from indigenous fermented condiments in Nigeria. However, the present 28-month laboratory study reported high recovery rates of coliforms and indicator bacteria in the fermented condiments analyzed in this study. The Gram-negative indicator bacterial isolates from fermented condiments in this study are similar to those of previous studies, which reported that *Alkaligenes viscolactis, Cory-*

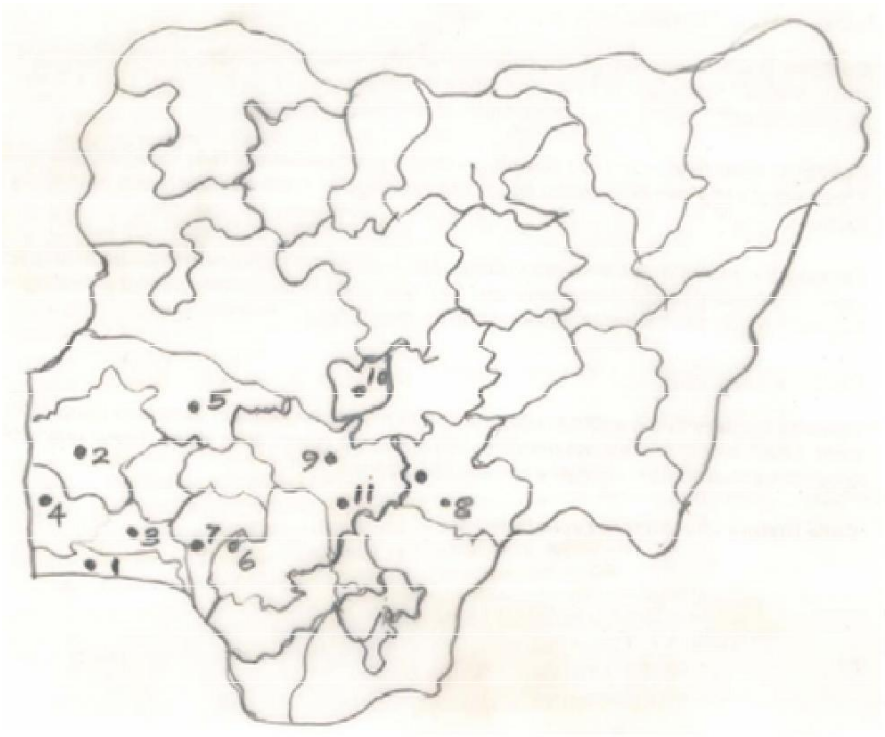

Figure 1. Sampling sources of the analysed condiments
Keys: 1 = Lagos, 2 = Ibadan, 3 = Ijebu-Ode, 4 = Abeokuta, 5 = Ilorin, 6 = Benin, 7 = Ore, 8 = Gboko, 9 = Lokoja, 10 = Abuja, 11 = Okenne

nebacterium spp*., Enterobacter cloacae* and *Pseudomonas* sp. were isolated from fermenting *P macrophylla* seeds during the production of *ukpaka* (Barber et al., 1988). *Pseudomonas* sp. was also isolated from fermenting castor oil seeds (*Ricinus communis*) for the production of *ogiri* (Barber *et al*., 1988; Odibo et al., 1992). Other microorganisms isolated infrequently and at very low numbers from fermenting *ogiri* include *Proteus* spp. and *E. aerogenes*. Some of the microorganisms associated with the fermented condiments include *Enterobacter cloacae* from fermenting *Prosopis* seeds for the production of *ogiri-okpei* (Odibo et al., 1992; Ogunshe et al., 2007; 2008), and *Pseudomonas* sp., *Proteus* sp*., Klebsiella* and *E*. *coli* from fermenting *iru* samples (Campbell – Platt, 1984; Ogbadu and Okagbue, 1988).

Food microbiologists are usually interested in the determination and studies on microbial flora of industrial importance, especially in selection of starter cultures for fermented foods; including fermented condiments. Most previous studies were also mainly on investigating the nutrient composition of the fermented condiments. However, the relatively high recovery of Gram-negative bacterial indicators and mannitol-fermenting, coagulase-positive *S. aureus* are of clinical importance. Staphylococci have been known worldwide to cause food-borne intoxications and poisonings (Klipstein et al., 1977; Brooks et al., 1998; Prescott et al., 2005). There is virtually no documented information available on the involvement of *S. aureus* in food-borne disease outbreaks of fermented food condiments origin, due to a virtually non-existing reporting system in Nigeria (Onuorah et al., 1987). *E. coli* that was recovered in significant rates from fermented food condiments in this study has become a significant public health concern with a worldwide distribution (Mead et al., 1999). Other Gram-negative bacteria isolated in this study have also been implicated in acute bacterial diarrhoeas and food poisonings (Klipstein et al., 1977; Onuorah et al., 1987). Enterotoxigenic gastroenteritis-causing genera such as *Pseudomonas*, *Enterobacter*, *Klebsiella* and *Proteus*, also isolated from the fermented condiments in this study, have been previously implicated as opportunistic pathogens and have become of increasing importance (Salyers and Whitt, 1994; Prescott et al., 2005), while *Citrobacter* sp. was also established by Sakazaki (Sakazaki, 1984) as an opportunistic pathogen.

The fermented condiments preparation is still a traditional family art done in households and the fermentations that do not require conscious introduction of the microbial flora into the fermenting environment (Ogbadu and Okagbue, 1988), thereby leading to the relative significant recovery of these indicator bacteria from the fermented condiments as confirmed by this study. The high recovery rates of *S. aureus* and total coliform in the fermented condiments indicate the unwholesomeness of the fermented condiments, which may result in an

increased risk of transmission of diseases to the humans who consume them.

Estimates of food-borne disease deaths are subject to uncertainty because the number of deaths caused by unidentified pathogenic agents in the food supply is unknown. However, in the influential study of food-borne disease in the United States by Mead et al. (1999) it was estimated that unknown food-borne agents caused 3 400 deaths per year, or 65% of the estimated 5 200 annual deaths from food-borne disease. No matter how alarming this estimate from a developed country like the United State may be, more alarming would be the estimates from developing countries like Nigeria. For decades, food microbiologists have developed various effective methods of food protection. However, the constant deve-lopment of multi-facet food processing technologies and the emergence of potent food-borne pathogens com-promised the efficacy of many antimicrobial interventions. Most technologies also fail to address the problem of bacterial debris remaining on the food surface. Further-more, some bacteria have the ability to develop resis-tance to antimicrobial interventions. All such factors contribute to the continuously growing concern of keeping our food safe.

A common source of food-borne disease is bacterial contamination of foods by food handlers. However, the safety aspects of fermented condiments are not ade-quately documented and appreciated in developing coun-tries like Nigeria (Ogunshe et al., 2007). It was generally observed that the water samples usually used in rinsing the boiled bean cotyledons prior to fermentation were highly polluted. It is a common practice among the Nigerian elites to wash the fermented cotyledons in clean water before adding to culinary, due to sandy mouth feel usually encountered while chewing such prepared foods; meanwhile, the portion washed off are the nutritious por-tion of the condiments. There therefore is need for pro-ducer and consumer education about the safety of the indigenous fermented condiments.

The pathogenicity and antibiotic susceptibility spectrum, and source(s) of the Gram-negative indicator bacteria in the fermented condiments are under investigation in our laboratories. The process control and non-chemical preservation and storage that can be simulated both in the cottage and industrial productions are also under study. These studies should yield results that may lead to improvement in process efficiency, enhance product quality and extend the shelf-life of these popular locally processed fermented-food products.

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REFERENCES

Azokpota P, Hounhouigan DJ, Nago MC (2006). Microbiological and chemical changes during the fermentation of African locust bean (*Parkia biglobosa*) to produce *afitin*, *iru* and *sonru*, three traditional condiments produced in Benin. Int J. Food Microbiol. 107 (3): 304-309.

Baird-Parker AC (1994). Foods and microbiological risk. Int. J. Food Microbiol. 140: 687-695.

Barber L, Achiehwu SC, Ibiawa EA (1988). Food Microbiology, 4th Ed., Acad. Press. New York. pp. 177- 182,

Brooks GF, Butel JS, Morse SA (1998). Medical Microbiology. 21st ed. Norwalk. Conn. Appleton & Lange, USA. p. 740

Buckenhuskes HJ (1993). Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. FEMS Microbiol. Rev. 12: 253 - 273.

Campbell–Platt G (1984). Traditional West African Foods. In Proceedings of Twentieth Anniversary Conference. Institute of Food Science and Technology (Britain) on Ethnic food Symposium. Proceeding of the Inst. Food Sci. Technol. 17: 214 - 218.

Chang GW, Brill J, Lum R (1989). Proportion of -D- glucuronidase-negative *Escherichia coli* in human faecal samples. Appl. Microbiol. 55: 335 - 339.

Cheesbrough M (1998). District Laboratory Practice in Tropical Countries. Part 1. UK: Cambridge University Press. P. 434

Cheesbrough M (2000).District Laboratory Practice in Tropical Countries. Part 2. UK: Cambridge University Press. p.454

Ejiofor MAN, Oti E, Okafor JC (1987). Studies on the fermentation of seeds of the African oil bean tree (*Pentaclethra macrophyla*). The International Tree Crops. 4: 135 - 144.

Harrigan WF, McCance ME (1976). *Laboratory methods in food and dairy microbiology*. Pp. 261-262, Acad. Press. London. New York p.342

Hesseltine CW (1965). A millennium of fungi, food and fermentation. Mycol. 57: 149-197.

Holding AJ, Collee JG (1972). Routine biochemical tests. *In*: Methods in microbiology. (6): 2 -32.

Jay JM (1993). Indices of food sanitary quality: microbiological standards and criteria, *In*. Modern food microbiology. 3rd ed. CBA Publishers Delhi, India, pp.409-435.

Klipstein FA, Engert RA, Short HB (1977). Relative enterotoxigenicity of coliform bacteria. J. Infect. Dis. 136: 205-215.

Leejerajumnean A, Ames JM, Owens JD (2000). Effect of ammonia on the growth of *Bacillus* species and some other bacteria. Lett. Appl. Microbiol. 30: 385-389.

Mead PS, Stusker L, Dietz V, McCraig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999). Food related illness and death in the United States. Emerg. Infect. Dis. 5: 607–25.

Obeta JAN (1982). A note on the microorganisms associated with the fermentation of seeds of the African oil bean tree (*Pentaclethra macrophyla*). J. Appl. Bacteriol. 54: 5433-5435.

Odibo FJC, Ugwu BA, Ekeocha OC (1992). Microorganisms associated with the fermentation of *Prosopis* seeds for *ogiri okpei* production. J. Food Sci. Technol. 29: 306-307.

Odunfa SA (1985). Biochemical changes in fermenting African locust bean (*Parkia biglobosa*) during *iru* fermentation. J. Food Technol. 20: 295 - 303.

Ogbadu LJ, Okagbue RN (1988). Bacterial fermentation of soy bean for *daddawa* production. J. Appl. Bacteriol. 65: 353-356.

Ogunshe AAO, Ayodele AI, Okonko IO (2006). Microbial studies on *aisa*, a potential indigenous laboratory fermented food condiment from *Albizia saman* (Jacq.) F. Mull. Pakist. J. Nutr. 5(1): 51-58.

Ogunshe AAO, Omotoso MO, Ayansina ADV (2007). Microbial studies and biochemical characteristics of controlled fermented *afiy*o- a Nigerian fermented food condiment from *Prosopis africana* (Guill and Perr.) Taub Pakist. J. Nutr. 6 (6): 620-627.

Ogunshe AAO, Jayeola AA, Ogundimu TC (2008). Microbial studies on

laboratory fermentation of *iregi* - a potential food condiment from *Delonix regia* (Boj. ex Hook.) Raf FOOD. 2 (1): 61-64.

Oguntoyinbo FA, Sanni AI, Franz CMAP, Holzapfel WH. (2007). *In vitro* fermentation studies for selection and evaluation of *Bacillus* strains as starter cultures for the production of *okpehe*, a traditional African fermented condiment. Int. J. Food Microbiol. 113 (2): 208-218.

Onuorah SI, Adesiyun AA, Adekeye JO (1987). Occurrence of staphylococci and coliforms in *Kunun zaki* and utensils used in its preparation in Samaru, Zaria. J. Food Agric. 1: 3 -34.

Prescott LM, Harley JP, Klein DA (2005). *Microbiology*, 6th edition. McGraw-Hill Inc. New York, USA.pp. 799-818

Sakazaki R (1984). *Citrobacter*. In *Bergey's Manual of Systematic Bacteriology*. Krieg NR, Holt JG (Eds.). Williams and Wilkins, Baltimore and London. ,pp. 458-461

Salyers AA, Whitt DD (1994). *Bacterial Pathogenesis:* A molecular approach, Washington, D.C., ASM Press, USA.

Sanni AI, Ogbonna DN (1991). The production of *owoh* — a Nigerian fermented seasoning agent from cotton seed (*Gossypium hirsutum* L.) Food Microbiol. 8(3): 223-229.

Sarkar PK, Cook PE, Owens JD (1993). *Bacillus* fermentation of soybeans. W. J. Microbiol. Biotechnol. 9: 295-299.

Seeley HW, Van Denmark PJ (1972). *A laboratory manual of Microbiology*. P. 361. 2nd edtn. Freeman and Co. San Francisco, USA.

Steinkraus KH (1991). African alkaline fermented foods and their relation to similar foods in other parts of the world. In: *Traditional African Foods*: *Quality and nutrition*. Eds. 87-92. Marcel Dekker, Wesby A, Reilly PJA, New York, USA

Whitaker JR (1978). Biochemical changes occurring during the fermentation of high protein foods. Food Technol. 32:175.